Computer-Aided 2D and 3D quantification of human stem cell fate from in vitro samples using Volocity high performance image analysis software.

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Public Summary:
Human stem cells have potential to be used for treatment of multiple diseases and trauma. To better understand the properties of stem cells and the ability of these to repair human conditions, many laboratories are routinely culturing and differentiating stem cells on a dish. These in vitro cultured stem cells are commonly classified and quantified from 2D or 3D images, and different image acquisition platform types used to collect the data have their advantages. Confocal microscopy is a common tool for image acquisition of z-stacks of optical slices of cells or tissues after immunohistochemistry, and it has the advantage of allowing for numerous adjustments, e.g. aperture size, image dimension, and scan speed that affect image quality, the rate of image capture, and file size. These parameters define not just the quality of 3D images, but also the time needed for image capture and the size of data files, which matters especially when dealing with the large number of samples common when testing effects of variable culture conditions such as small molecules and drugs on stem cell differentiation. Accordingly, the numbers of samples processed for fate analysis are increasing exponentially, and accurate automated cell fate analysis from 2- and 3-dimensional (2D-3D) images would be an enormous improvement in this field. Conventionally used manual image analysis of stem cell fate has a low validity, and development of an accurate and precise tool that reduces variability and the time needed for human stem cell fate analysis will improve productivity and interpretability of the data across research groups. In this publication, we have created protocols for high performance image analysis software Volocity® to classify and quantify cytoplasmic and nuclear cell fate markers from 2D-3D images of in vitro differentiated human neural stem cells. The protocols can be modified to suit a specific user's needs. They allow for semi-automated, more time efficient and accurate stem cell fate classification and quantification, although data verification by operator is still highly recommended. The software based, operator validated, stem cell fate classification and quantification is 2-fold faster than manual image analysis, and yields the highest ≥ 94.4% correspondence with human recognized objects.

Scientific Abstract:
Accurate automated cell fate analysis of immunostained human stem cells from 2- and 3-dimensional (2D-3D) images would improve efficiency in the field of stem cell research. Development of an accurate and precise tool that reduces variability and the time needed for human stem cell fate analysis will improve productivity and interpretability of the data across research groups. In this study, we have created protocols for high performance image analysis software Volocity(R) to classify and quantify cytoplasmic and nuclear cell fate markers from 2D-3D images of human neural stem cells after in vitro differentiation. To enhance 3D image capture efficiency, we optimized the image acquisition settings of an Olympus FV10i(R) confocal laser scanning microscope to match our quantification protocols and improve cell fate classification. The methods developed in this study will allow for a more time efficient and accurate software based, operator validated, stem cell fate classification and quantification from 2D and 3D images, and yield the highest >/=94.4% correspondence with human recognized objects.